

CLAIMS

What is claimed is:

1. A genetic vector for stable transfection and expression of a desired protein  
5 within eukaryotic cells comprising:
  - (a) distal 5' flanking sequences of a eukaryotic locus;
  - (b) proximal 5' regulatory sequences of a eukaryotic locus;
  - (c) at least a first insertion site for a first heterologous coding sequence; and
  - (d) proximal 3' regulatory sequences effective for transcription termination of a  
10 eukaryotic locus;wherein said sequences are operably joined in order (a)-(d) in a 5' to 3'  
orientation, with optional linker sequences between adjacent sequences; and  
wherein
  - (1) said distal 5' flanking sequences comprise a sequence of at least 100 bases  
15 having at least 70% identity to a nucleotide sequence found between 20 bp and 100,000  
bp 5' of a transcriptional initiation site of a ferritin heavy chain locus; or
  - (2) said proximal 5' regulatory sequences comprise a sequence of at least 20  
bases having at least 70% identity to a nucleotide sequence found between 1 bp and  
10,000 bp 5' of a translational initiation codon of a ferritin heavy chain locus.  
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2. A genetic vector for stable transfection and expression of a desired protein  
within eukaryotic cells comprising:
  - (a) distal 5' flanking sequences of a eukaryotic locus;
  - (b) proximal 5' regulatory sequences of a eukaryotic locus;
  - 25 (c) at least a first heterologous coding sequence encoding said desired protein;  
and
  - (d) proximal 3' regulatory sequences effective for transcription termination of a  
eukaryotic locus;wherein said sequences are operably joined in order (a)-(d) in a 5' to 3'  
30 orientation, with optional linker sequences between adjacent sequences; and  
wherein

(1) said distal 5' flanking sequences comprise a sequence of at least 100 bases having at least 70% identity to a nucleotide sequence found between 20 bp and 100,000 bp 5' of a transcriptional initiation site of a ferritin heavy chain locus; or

5 (2) said proximal 5' regulatory sequences comprise a sequence of at least 20 bases having at least 70% identity to a nucleotide sequence found between 1 bp and 10,000 bp 5' of a translational initiation codon of a ferritin heavy chain locus.

10 3. A genetic vector as in any one of claims 1-2 wherein said distal 5' flanking sequences are derived from a ferritin heavy chain locus.

4. A genetic vector as in any one of claims 1-2 wherein said proximal 5' regulatory sequences are derived from a ferritin heavy chain locus.

15 5. A genetic vector as in any one of claims 1-2 wherein said proximal 5' regulatory sequences and said distal 5' flanking sequences are derived from a ferritin heavy chain locus.

20 6. A genetic vector as in any one of claims 1-5 wherein said proximal 3' regulatory sequences are derived from a ferritin heavy chain locus.

7. A genetic vector as in any one of claims 1-6 further comprising distal 3' flanking sequences of a ferritin heavy chain locus.

25 8. A genetic vector as in any one of claims 1, and 3-7 wherein said insertion site for a heterologous sequence includes at least one restriction endonuclease site.

9. A genetic vector as in claim 8 wherein said insertion site for a heterologous sequence is a polylinker site including at least two restriction endonuclease sites.

30 10. A genetic vector as in any one of claims 1-9 wherein said proximal 5' regulatory sequences include a eukaryotic intron sequence.

11. A genetic vector as in claim 10 wherein said eukaryotic intron sequence is derived from intron 1 of a ferritin heavy chain gene.
12. A genetic vector as in any one of claims 1-11 wherein said proximal 5' regulatory sequences include untranslated exon sequences.
13. A genetic vector as in any one of claims 1-12 wherein said distal 5' flanking sequences and said proximal 5' regulatory sequences have a total length of between 1,000 and 10,000 bases.
14. A genetic vector as in any one of claims 1-12 wherein said proximal 3' regulatory sequences and any distal 3' flanking sequences have a total length of between 1,000 and 10,000 bases.
15. A eukaryotic cell transfected with a vector of any one of claims 1-14.
16. A eukaryotic cell as in claim 15 wherein said vector has stably integrated into a chromosome of said cell.
17. A eukaryotic cell as in any one of claims 15-16 wherein said first coding sequence is expressed in said cell.
18. A eukaryotic cell comprising
- (a) distal 5' flanking sequences of a eukaryotic locus;
  - (b) proximal 5' regulatory sequences of a eukaryotic locus;
  - (c) at least a first coding sequence; and
  - (d) proximal 3' regulatory sequences effective for transcription termination of a eukaryotic locus;
- wherein said sequences are operably joined in order (a)-(d) in a 5' to 3' orientation, with optional linker sequences between adjacent sequences; and wherein

(1) said distal 5' flanking sequences comprise an exogenous sequence of at least 100 bases having at least 70% identity to a nucleotide sequence found between 20 bp and 100,000 bp 5' of a transcriptional initiation site of a ferritin heavy chain locus; or

5 (2) said proximal 5' regulatory sequences comprise an exogenous sequence of at least 20 bases having at least 70% identity to a nucleotide sequence found between 1 bp and 10,000 bp 5' of a translational initiation codon of a ferritin heavy chain locus.

19. A eukaryotic cell comprising:  
an exogenous 5' distal flanking sequence derived from a ferritin heavy chain  
10 locus operably joined to a coding sequence.

20. A method of producing a desired protein in a eukaryotic cell comprising:  
(a) providing at least one cell of any one of claims 15-19 or a descendent thereof;  
15 (b) maintaining said cell in a culture under conditions which permit high expression of said desired protein; and  
(c) isolating said desired protein from said culture.